EXHIBIT "D"

1: Acta Virol. 2006;50(1):25-32.

Influence of guanidine on proteinase K resistance in vitro and infectivity of scrapie prion protein PrP(Sc).

Gao JM, Zhou XB, Xiao XL, Zhang J, Chen L, Gao C, Zhang BY, Dong XP.

State Key Laboratory for Infectious Disease Prevention and Control, National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Ying-Xin Rd. 100, Beijing 100052, P.R. China.

As the scrapie prion protein PrP(Sc) is rich in beta-sheets it aggregates into prion rods, which show infectivity and proteinase K (PK) resistance. Consequently, dissociation of prion rods and breakdown of beta-sheets in PrP(Sc) by denaturation results in loss of both infectivity and PK-sensitivity. In this study, the effects of guanidine (Gdn), which solubilizes and denatures proteins by breaking down their higher structure, on the solubility, the PK-resistance in vitro and the infectivity of PrP(Sc) of scrapie strain 263K was examined. The infectivity was assayed by intracerebral inoculation into hamsters. Brain tissues of scrapie-infected hamsters were used for preparation of homogenates and crude extracts of PrP(Sc). A treatment of PrP(Sc) with Gdn enhanced its PK-sensitivity in a dose-dependent manner. The PK-resistance in vitro of PrP(Sc) denatured with lower concentrations of Gdn (<2.5 mol/l) could partially resume by renaturation. Gdn markedly reduced or, at higher concentrations, even destroyed the infectivity of PrP(Sc). On the other hand, the infectivity of PrP(Sc) inactivated by denaturation could be partially restored by renaturation. These results confirmed our assumption that all the alternations in the PK-resistance and the infectivity of PrP(Sc) caused by Gdn resulted from changes in its higher structure. However, it should be emphasized that a complete loss of PK-resistance of PrP(Sc) may not necessarily mean its full non-infectivity.

PMID: 16599182 [PubMed - indexed for MEDLINE]